The enzymatic vharacteristics and active formation of the luminous enzyme from the marine ostracod.

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Luminous crustacean *Vargula hilgendorfii* and *Cypridina noctiluca* are commonly known as the sea-firefly, and are found around the coast of Japan. The bioluminescences of these creatures are highly enzyme specific reaction with the luciferin, luciferase and molecular oxygen. Recently, *V.hilgendorfii luciferase* was only cloned and its gene was applied for studying the gene expression as a sensitive reporter enzyme. However, the knowledge of luciferase is limited, like the active formation of protein and the function of the glycochain were still not well established. In this study, to understand the bioluminescence mechanism in the sea-firefly luciferase, new purification procedures and characterization of native *V.hilgendorfii* luciferase were done. While, cloning and characterization of *C.noctiluca* luciferase were also performed.

Native *V.hilgendorfii* luciferase is a glycoprotein, which has a molecular mass of 61.9 kDa and is heat stable. On the other hand, *C.noctiluca* luciferase consisted of 553 amino acid residues with molecular weight 61.4 kDa deduced from the nucleotide sequence. *C.noctiluca* luciferase shows amino acid identity of 83.1% with that of V.hilgendorfii luciferase. *C.noctiluca* luciferase is also a glycoprotein with two putative N-linked glycosylation sites. To confirm the role of the glycochain, *C.noctiluca* luciferase was deglycosylated by N-glycosidase F, but the level of activity remained unchanged. Alternatively, when Asn-182 was replaced to Asp by site-directed mutagenesis, expressed luciferase was dramatically decreased. These results strongly suggest that the glycochain bound to Asn-182 could be involved in protein stability, but not in the enzymatic activity.